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by Sri Puji Astuti Wahyuningsih

Submission date: 16-Nov-2018 04:07PM (UTC+0800)
Submission ID: 1040211054
File name: Bukti_C18.pdf (422.23K)
Word count: 3637
Character count: 18888

Polysaccharide Krestin Activity of *Coriolus versicolor* Extract on Interleukin-12 Level of *Mus muculus* Exposed to *Mycobacterium tuberculosis*

Sri Puji Astuti Wahyuningsih[1], Sugiharto[1], Nurul Wiqoyah[2] & Zakiyatun Nuha[1]

[1]Depart. of Biology, Faculty of Science and Technology, Airlangga University, Surabaya 60115 Indonesia
[2] Microbiology Laboratoty, Faculty of Medicine, Airlangga University, Surabaya 60115 Indonesia
Email: sri-p-a-w@fst.unair.ac.id sri-p-a-w@fst.unair.ac.id

Abstract. This study aimed to determine the effect of polysaccharide krestin (PSK) of *Coriolus versicolor*'s extract on IL-12 levels in the *Mus musculus*'s blood serum that had been exposed by *Mycobacterium tuberculosis*. This study used a completely randomized design were divided into six treatment groups. They were K (control group without treatment), K+ (positive control by providing PSK), K- (negative control with exposed *M. tuberculosis*), P1 (treatment using PSK before exposured by *M. tuberculosis*, P2 (treatment using PSK after exposured by *M. tuberculosis*), and P3 (treatment using PSK before and after exposured by *M. tuberculosis*). PSK given concentration was 200 mg/kg bw, while the number of bacteria for exposure was 0.25 Mc Farland with double exposure. Each treatment there were four replicates. Blood serum of mice were isolated and measured levels of IL-12 by ELISA kit. Data analysis used Brown Forsythe test. The results showed that the highest levels of IL-4 was K-. PSK administration in the treatment of P1, P2, and P3 showed the levels of IL-12 not significant with the K and K +. Conclusion of the study was the treatment using PSK had no effect on the level of IL-12 in *Mus muculus* exposed by *M. tuberculosis*.

Keywords: Coriolus versicolor*; interleukin-12, polysaccharides krestin;* Mycobacterium tuberculosis.

1 Introduction

Tuberculosis or TB is an infectious bacterial disease caused by *Mycobacterium tuberculosis*, which most commonly affects the lungs. It is transmitted from person to person via droplets from the throat and lungs of people with the active respiratory disease. The symptoms of active TB of the lung are coughing, sometimes with sputum or blood, chest pains, weakness, weight loss, fever and night sweats [Departemen Kesehatan RI, 7].

In healthy people, infection with M. tuberculosis Often causes no symptoms, since the person's immune system acts to "wall off" the bacteria. Tuberculosis can be treated with antibiotics for 6 months in a row. It is important to look for other alternative materials that can be used to enhance the immune response to tuberculosis.

Medicinal mushrooms have an established history of use in traditional oriental therapies. Modern clinical practice in Japan, China, Korea, and other Asian countries continues to rely on mushroom. Mushrooms effects have been demonstrated for many including extracts of species from *C. versicolor* [Ooi & Liu, 14].

It is well established that many mushroom-extracted compounds are commonly used as immunomodulators or as Biological Response Modifiers (BRM). The basic strategy underlying immunomodulation is to identify aspects of the host response that can be enhanced or suppressed in such a way as to augment or complement a desired immune response. Whether certain compounds enhance or suppress immune responses depends on a number of factors, including dose, route of administration, timing of administration of the compound, mechanism of action, and site of activity. Knowledge of the specific components of cytokine networks and signaling pathways and their role in the regulation of immune responses is important in designing strategies to augment these responses.

C. versicolor extract can increase the number of leukocytes, macrophages and spleen weight [Wahyuningsih, 20], the provision of PSK from *C. versicolor* increase the number of immunocompetent cells, increase immune response non-specific and specific due to infection with M. tuberculosis (Wahyuningsih *et al.*, 21). The active compounds contained in mushrooms is β-glucan [Guggenheim, 9; Moradali *et al.*, 13].

β-Glucan is known to stimulate the formation of pro-inflammatory mediators such as complement components, interleukin 1 (IL-1), tumor necrosis factor (TNF-α), interleukin 2 (IL-2) and eicosanoids [Yu *et al.*, 27]. β-Glucan increases the production of IL-2, which stimulates the differentiation of B cells activated [Vetvicka *et al.*, 24]. In this study, administration of PSK done in three different time ie before exposure, after exposure and before and after exposure to *M. tuberculosis*. Giving PSK before exposure to *M. tuberculosis* can serve as a preventative that will encourage the formation of antibodies so the body does not easily infected. Giving PSK after exposure was conducted to determine the effectiveness of the PSK in eliminating acid-resistant bacteria. Giving PSK before and after exposure to M. tuberculosis as a preventive and curative efforts that encourage the formation of antibodies [Wahyuningsih *et al.*,

22]. Pietro [15], β-glucan is effective for the prevention and treatment of diseases related to the resilience of the body's immune system.

B-glucan compounds of PSK related to the primary receptor of the immune system that is Dectin-1, Toll-Like Receptor 2/6 (TLR-2/6) and Complement Receptor 3 (CR3). Dectin-1 known to play a role as a co-stimulator of T cells and is the receptor that is expressed by macrophages [Willment, 23]. In addition Dectin-1 can work with TLRs to induce and increase the production of cytokines [Gantner *et al.*, 8). Interleukin-12 binding to its receptor activates the tyrosine kinase 2 (Tyk2) and janus kinase 2 (JAK2). This causes the phosphorylation of tyrosine residues of the signal transducer and activator of transcription 3 and 4 (STAT3 and STAT4). The occurrence of tyrosine phosphorylation process is responsible for the formation of STAT4 / STAT4 homodimer and STAT3 / STAT4 heterodimers. Both dimers are experiencing translocation into the nucleus and binds to the IFN-γ gene promoter [Thierfelder *et al.*, 19]. Promoter activation in the IFN-γ gene resulted in the synthesis of proteins that express IFN-γ protein.

Along with the growing understanding of the body's immune response in the face of infection, the more developed also research into components that can affect the immune response. If PSK can increase the levels of IL-12 in the blood, the immune system can increase too. If it is proven that extracts of *C. versicolor* can be used as a natural immunostimulatory to inhibit the growth of M. tuberculosis and reduce the number of TB patients. Active TB disease is estimated to have reached 1.9 billion, and it can prevent transmission because only people with active TB can transmit the disease.

2 Materials and Methods

2.1 Preparation research

Thirty female mice aged 8-10 weeks strain Balb/C acclimatized for one week and are grouped into six, namely K (only given distilled water), K- (exposure to *M. tuberculosis* only), K + (given PSK only), P1 (PSK was given before exposure to *M. tuberculosis*), P2 (PSK administered after exposure to *M. tuberculosis*), P3 (PSK given before and after exposure to *M. tuberculosis*). Feed and water provided ad libitum.

2.2 Isolation and Measurement Levels PSK

Coriolus versicolor were collected from Kediri, Tulungagung and Surabaya. Fungi identified, wind dried and pulverized to form a coarse powder. Then it

was extracted by the method of Cui & Cristi [5] and Cui *et al*. [6] as modified by Wahyuningsih *et al*. [20] PSK levels determined by the method of phenol-sulfuric acid assay. PSK dose used was 200 mg/Kg body weigth.

2.3 Treatment research

Giving PSK done for 7 days. Exposure to M. tuberculosis performed twice with an interval of two weeks via intraperitoneal. The number of bacteria is 0.5 Mc Farland.

2.4 Isolation serum

Blood was drawn through intracardia. Blood left in a tilted position at room temperature for three hours to form two phases, namely the colored translucent top and the bottom is red. Then, it centrifuged 3000 rpm, 10 min at 4 ° C. Serum or supernatant portion was taken.

2.5 Immunosrbent-Like Enzyme Assay (ELISA)

Detection of IL-12 used to a sandwich ELISA (Koma Biotech ELISA Kit IL-12). Levels of IL-12 was read by ELISA reader at a wavelength of 450 nm.

2.6 Data analysis

Data were tested with the Kolmogorov-Smirnov test and homogeneity of variance. Then analyzed by Brown Forsythe at the level of 5% ($\alpha = 0.05$).

3 Result

Levels of Interleukin 12 in the blood serum is a picture of an immune response as a result of exposure to M. tuberculosis were shown in Table 1. The results of the ELISA is a yellow color reaction. This color is obtained after a complete bond between the antigen, primary antibody and enzyme-labeled secondary antibody.

The results showed that average levels of IL-12 in the control group (K) was 25.91 ± 11.08 (pg / mL). Positive control group (K +) was 26.37 ± 11.49 (pg / mL). Negative control group (K) was 37.25 ± 27.73 (pg / mL). P1 group was 31.85 ± 16.22 (pg / mL). P2 group was 31.43 ± 10.47 (pg / mL). P3 group was 26.07 ± 10.99 (pg / mL). Kolmogorov-Smirnov test showed a significance level of 0.068. This indicates that the data were normally distributed. Homogeneity test showed a significance level of 0,010. This indicates that the data has a

variance that is not homogeneous. Data were analyzed by Brown Forsythe test to determine the effect of treatment in each group. The test results showed a significance level of 0.828. This shows that there was no effect of feeding time on levels of IL-12 in mice blood serum as a result of exposure to *M. tuberculosis*.

Table 1 Levels of IL-12 after administration of PSK from *C. versicolor* in mice exposed to *M. tuberculosis*

Treatment	Levels of IFN-γ on replay to				Mean ± SD (pg/mL)
	1	2	3	4	
K	24.90	30.75	27.32	20.68	25.91[a] ±11.08
K+	30.44	31.07	23.28	20.68	26.37[a] ±11.49
K-	81.88	26.90	19.84	20.36	37.25[a] ±27.73
P1	33.57	48.90	23.04	21.89	31.85[a] ±16.22
P2	24.77	32.05	45.97	22.93	31.43[a] ±10.47
P3	21.22	26.90	29.66	26.48	26.07[a] ±10.99

4 Discussion

In the study used an indicator to determine the levels of cytokine immune response to infection with M. tuberculosis and also the effect of PSK. Cytokines are small proteins as a mediator and regulator of immunity, inflammation and hematopoesis. The cytokines produced in response to the stimulus of the immune system. Cytokines works by binding to specific membrane receptors, which then carry signals into the cell via second messengers (tyrosine kinase), to alter gene expression (Judarwanto, 11). Cytokines produced in the body when the immune response as a result of infectious microorganisms or foreign substances that enter the body (Romagnani, 16). Type of cytokine that is used as an indicator in this study is the production of Th1 cytokines, namely IL-12. Interleukin is a cytokine that acts against leukocytes, while interferon is a type of cytokine that plays a role due to interference virus (Lewis, 12).

Tuberculosis is a disease caused by intracellular bacterial pathogens, namely *M. tuberculosis* (Higuchi et al., 10). One type of cytokine that plays an important role in the defense against intracellular pathogens such as *M. tuberculosis* is IL-12. APC cells produce IL-12 when stimulated by lypotechoat acid and peptidoglycan. Two components are bacterial cell wall constituent. Wall of *M. tuberculosis* can induce macrophages to produce IL-12 (Yoshida & Koide, 26).

In this study, *M. tuberculosis* acts as an antigen. The bacteria will induce the secretion of IL-12. PSK act as immunostimulatory which is expected to enhance the immune response due to infection with *M. tuberculosis*. Polysaccharides krestin from *C. versicolor* extract known to contain β-glucan compounds. The compounds may be associated with the primary receptor of the immune system such as Dectin-1, Toll-Like Receptor 2/6 (TLR-2/6) and Complement Receptor 3 (CR3). Dectin-1 acts as a co-stimulator of T cells and is the receptor that is expressed by macrophages (Willment, 23).

In Table 1 it can be seen that the levels of IL-12 between the control and treatment groups showed no significant difference. However, if compared to K, then the levels of IL-12 on K +, K-, P1, P2, and P3 showed an increase. Levels of IL-12 highest detected in the K group treated only exposure to *M. tuberculosis*. It shows that there are components of antigens on the cell wall of bacteria that can enhance the immune response. According to Crick *et al*. (4), TB bacterial cell wall composed of mannose-capped lipoarabinomannan (ManLAM), lipomannan (LM), phosphatidyl-myo-inositol mannosides (PIMs), arabinomannan, mannan and Manno-glycoproteins. It is a strong antigen to induce an immune response. Through TLRs, *M. tuberculosis* cell wall components associated with lipoprotein so as to induce the production of IL-12 (Brightbill *et al*., 2).

PSK was known to induce the body's immune response, but the strength of induction was weaker when compared to components of M. tuberculosis antigens. It can be seen from Table 1, which showed that levels of IL-12 to K + is higher when compared with K. Levels of IL-12 in the group P1, P2, and P3 showed lower than K-. This was probably related to the role of β-glucan. Glucan can bind directly to specific receptors of immune cells causing immunomodulating effects (Vos *et al.,* 25). This means it can increase or decrease the immune response. But the mechanism of action of β-glucan as immunomodulators, especially in *M. tuberculosis* infection is not known.

Statistically, the provision PSK does not affect the levels of IL-12. Most likely behind it was the existence of mechanisms mediated cascade of other cytokines stimulated due to the antigen of *M. tuberculosis*. Another possibility was happening related to the nature of cytokine that a type of cytokine can be produced by various types of cells and can cause different effects through various channels (Soeroso, 17). Various types of cytokine sometimes also have the same function for one type of cells (Abbas *et al*., 1). The redundancy properties that make it difficult to know the mechanism of action of one single type of cytokine.

β-Glucan contained in PSK of *C. versicolor* known to play a role as an immunomodulator that acts on the immune system of specific and non-specific. In the non-specific immune system, β-glucan helps to recognize the antigen and respond faster. Dectin-1 is a receptor type II transmembrane protein, can bind to β-1,3 and β-1,6 glucan that can do the initiation and regulation of the non-specific immune response (Brown *et al*., 3). Dectin-1 is expressed by immune cells that play a role in non-specific immune system and have been found on macrophages, neutrophils, and dendritic cells (Taylor *et al*., 18). In the specific immune response, Dectin-1 is able to activate T cells and induces the secretion of cytokines (Taylor *et al*., 18). According to Gantner *et al.* (8), Dectin-1 is able to induce and increase the secretion of cytokines through TLRs. In addition, the β-glucan known to be effective for the prevention and treatment of diseases associated with immune system resistance (Pietro, 15).

The conclusion from this study is the provision PSK dose of 50 mg/kg body weight had no effect on levels of IL-12 in mice blood serum as a result of exposure to *M. tuberculosis*. Further studies, using other indicators that can be known immune mechanisms of TB patients by administration of PSK from *C. versicolor* extracts.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9